THE INTRACELLULAR DEVELOPMENT OF A GREGARINE *FRENZELINA AMPELISCA* N. SP.

NADINE NOWLIN AND INEZ SMITH
University of Kansas

The parasites described below are found in the small Amphipod, *Ampelisca spinipes,* collected from the Eel Pond, Woods Hole, Mass., during August, 1912, and April, May, and August, 1914. Examinations of hosts were made at the dates mentioned through a series of more than two hundred carefully sectioned and stained slides, as well as on the living material, and few cysts were seen. That we have not yet found the season for their production is possible, but what is more probable is that only the vegetative stage is passed in Ampelisca and the spore stages in another host or free in the water. With this idea we have decided to publish what we have in the hope that someone may later complete the life-history.

The best fixation was found to be in Schaudinn's fluid, though Bouin's and sublimate-acetic were used satisfactorily. Sagittal sections, ranging in thickness from 7 to 25μ were stained with either Heidenhain's iron-hematoxylin or Mann's methylene blue-eosin mixture. Both methods gave good results.

The only part of the anatomy of Ampelisca which interests us here is that of the digestive tract and its glands. The alimentary canal consists of a straight tube from mouth to anus, divided into three general parts, stomadeum, mid-gut and proctodeum. The stomadeum and proctodeum, both of which are relatively short, are lined with a chitinous material, which in the stomadeum forms the masticatory stomach. The long, straight mid-gut makes up the principal part of the digestive tract. At its anterior and posterior ends it is lined with large epithelial cells of the columnar type, while in the central portion the epithelial cells are much more flattened. A very thin, delicate, non-chitinous cuticle, described in certain of the amphipods, is present over the digestive epithelium of Ampelisca.

The hepatic ceca, four in number, empty into the anterior end of the mid-gut. They are very long, two of them extending almost to the posterior end of the animal. The cells making up the hepatic ceca are large with characteristic vacuoles and large nuclei having karyosome-like nucleoli.

There are also a pair of small, pouch-like glands at the posterior end of the mid-gut and a single short dorsal cecum anterior to the junction of the hepatic ceca with the mid-gut.

Frenzelina ampelisca nov. spec.

The earliest stages of this parasite are intracellular, occurring in the digestive epithelium throughout the entire length of the mid-gut. In one host these very small intracellular forms were extremely numerous from the junction of the stomodeum with the mid-gut to the proctodeum, all stages of their growth being found. As there is often a pure infection of this form there is no danger of confusing it with two other gregarine inhabitants of Ampelisca.

Figures 1 and 2 represent two of these earliest forms, smaller than the nuclei of the epithelial cells. There is not a great deal of structure to be seen in them at this time. The protoplasm is more deeply stained and much less vacuolar than in the older stages. The nucleus is characteristically vesicular with a large, dark karyosome. No division of the body into protomerite and deutomerite is yet to be seen. The form represented in Figure 1 is 3.75 by 2.5μ.

The parasite is distinctly like the older form when it attains a size of 13.75μ by 5.55μ (Fig. 3). At this time it is usually very vacuolar, the cytoplasm having the appearance of a coarse reticulum. In this stage the body is always divided distinctly into protomerite and deutomerite, the protomerite being thick and short, from one-fifth to one-third the length of deutomerite. The cuticle is very thin and shows no signs of either cross or longitudinal striations or of myonemes. The nucleus is vesicular in form with a large, deeply staining karyosome, a distinct membrane and a homogeneous space between karyosome and membrane. A second small nucleus of the vesicular type with a deeply staining karyosome can usually be distinguished in the protomerite, even in very young forms (Figs. 4 and 6), occasionally a few unorganized chromatin granules are found in its place, and there are a few cases in which it seems actually to be lacking. Léger and Duboscq (1907) have described such a nucleus, which seems to be transitory and takes no part in reproduction in Pterocephalus.

The nucleus of the host cell is usually displaced by the young gregarine (Figs. 3 and 4), and as growth proceeds the parasite pushes out until the cell in which it was originally clearly located can no longer be distinguished. The gregarine makes for itself a considerable cavity among the epithelial cells (Figs. 5 and 7).

The nuclear structure of the parasite remains the same except for a proportional growth with the cytoplasm, but, on the other hand, the cytoplasm of the larger intracellular forms shows a marked change from that of the younger condition. This change is not always constant, however, for a given size of the parasite.

Figure 7 represents the largest intracellular form found, being 60.62 by 13.12μ. This form shows quite distinctly the change in cytoplasm,

which has become filled with large, clear granules instead of being reticular as in the early stage. These granules may be of the nature of stored food material for the reproductive process.

Figure 15 shows the not uncommon occurrence of this parasite in the cells of the hepatic ceca. Stages as small as those sometimes seen in the intestinal epithelium have not been found in the liver, nor have the large forms with the granular cytoplasm been found there. Figure 15 is a good example of the type found in the cells of the hepatic ceca. The protomerite and its nucleus are distinct as in the intestinal forms, and in every essential these gregarines resemble the intracellular stage in the intestinal epithelium with the exception of a greater tendency for the protomerite to be subdivided either once or twice (Figs. 15 and 16) giving a papillate appearance.

A few forms have been found free in the lumen of the mid-gut. With two exceptions these resemble the intracellular parasites of the same size, sometimes with a subdivision of the protomerite, a flattened knob, sometimes without.

Figure 8 represents one of the exceptions. In this form the cuticle presents a thickened appearance and is distinctly grooved longitudinally. The protomerite shows an indistinct division, beyond which the grooves do not extend. The cytoplasm is of the characteristic, mottled, reticulate type, and the nucleus typically vesicular but with four small patches of chromatin closely applied, at different points, to the membrane. There are two nuclear bodies in the protomerite of this specimen.

The other exception is a form similar to the one just described, but with the cuticle much thicker and quite shining. The grooves are also more conspicuous. The whole thing stains deeply and is too indistinct to show structural details.

A few free forms, similar in all respects to those found within the cells of the hepatic ceca, were found in the lumen of those glands. Figure 16 represents the biggest, thickest form found in the hepatic ceca. It is 43.75 by 18.75μ and is partly embedded at the protomerite end, in the cells of the gland. It differs from most forms by showing a deeply staining mass of granules surrounding the nucleus. Extracellular forms of this parasite are rare.

Young gregarines, no doubt, pass from the digestive tract into the hepatic ceca at the point where they join the mid-gut. They seem also to pass directly through the walls of the intestine and glands, into the coelom. Circumstantial evidence points to the latter as being at least one method by which exit from the intestine may take place. A number of cases of the intracellular forms in the intestine breaking through on the coelomic side of the wall have been observed. Figures 5 and 6 represent two forms, one just beneath the membrane on the coelomic

side of the intestinal wall, and the other apparently pushing its way out. These forms were found at the extreme anterior end of the mid-gut where the epithelium is quite thick. Still other forms were found more than half way through the wall in the central region of the mid-gut.

Only two examples of what is probably copulation were found (Figs. 11 and 12). This process takes place end to end. The sporonts (Fig. 11) are attached to the digestive epithelium at the anterior end of the mid-gut. This stage must follow very quickly upon that of the largest intracellular form (Fig. 7), for they differ in no essential detail from that form. The cytoplasm is filled with large, clear granules, and the nuclei are typically vesicular with a large, deeply staining karyosome.

Union takes place by the pushing of the protomerite of one form in the posterior end of the other. The outline of the protomerite containing a faint but evident second nucleus can be plainly seen within the body of the satellite. This copula is attached to the epithelial cells near the anterior end of the mid-gut. The method by which the sporonts join each other seems to be a sucker-like invagination on the posterior end of the primite. That there may be such an invagination before the two are united is shown in Figure 16.

A later stage of copulation was also found, and is represented by Figure 12. This pair occurred free in the lumen of the mid-gut. The difference in size of the sporonts is very noticeable, the primite measuring 62.5 by 15.62μ, the satellite 31.25 by 6.25μ. The protomerite of the satellite fits into an invagination of the posterior end of the primite. The protomerite is modified in each sporont and its nuclear body has lost the membrane, but the chromatin mass may be seen in each. Likewise the form of the deutomerite is changed from the earlier copula as seen in Figure 12. The cytoplasm contains many coarse granules and stains very deeply. The primite is much swollen and is vacuolar.

Léger and Duboscq (1909) found similar copula in *Frenzelina conformis* and designated them as old copulating pairs ready to form cysts. One cyst was found in the lumen of the intestine showing unequal sporonts (Fig. 12), but we cannot say certainly that it belonged to this parasite. Whether the cyst formation takes place in the intestine of this host can probably be determined by a study of Ampelisca later in the fall and winter. We have investigated them in spring and found none, infection even in the early trophozoite stage being slight at this time. Because cysts containing two copulants were found free in the water containing Ampelisca, and because so few old copula are found in the intestine we suspect that *Frenzelina ampelisca* is often shed with the feces during the copula stage and encysts in the water.

SUMMARY

A new species called ampelisca is added to the genus Frenzelina, created by Léger and Duboscq (1907) for certain crustacean gregarines.

The distinguishing character of this species are (1) smaller size than other species reported and (2) possession of a nucleus in the protomerite.

This is the second time intracellular development has been reported for Frenzelina, Watson's (1916) being the first report.

REFERENCES CITED

Léger, L., and Duboscq, O. 1907. L'evolution des Frenzelina (n. g.) Gregarines intestinals des crustacés décapods. C. R. acad. sci., Paris, 145: 773-4.

1909. Etudes sur la sexualité chez les grégarines. Arch. Protist., 17: 19-134, 5 pl.

1916. Watson, M. E. Three New Gregarines from Marine Crustacea. Jour. Parasitol., 2: 129-36, 1 pl.

Explanation of Plate

Frenzelina ampelisca

All drawings made to scale with Spencer 8 oc. and 1/12 oil immersion objective, except two diagrams, Figures 18 and 19.

Figures 1 to 13 show development of the parasite beginning with the earliest stage found in the intestinal epithelium.

Fig. 1.—Earliest intracellular stage found. 3.75 by 2.5μ.

Fig. 2.—Slightly larger stage. Still smaller than epithelial nucleus.

Fig. 3.—Young trophozoite growing inside epithelial cell of structure wall; shows protomerite; cytoplasm very vacuolar. 13.75 by 5.55μ.

Fig. 4.—Intracellular forms found near posterior end of intestine. Protomerite of one showing two nuclei; this sporont 15.61 by 6.25μ. Lower sporont 17.5 by 5.62μ.

Fig. 5.—Looking squarely on a form embedded in intestinal epithelium. Showing distinct nucleus in protomerite. Cytoplasm reticular. 28.75 by 9.1μ.

Fig. 6.—Apparently going through intestinal wall into colome. Upper 23.12 by 7.5μ; lower 20 by 8.12μ.

Fig. 7.—Largest intracellular form found, 60.62 by 13.12μ.

Fig. 8.—Free in lumen of intestine. Shows thick cuticle with definite longitudinal constrictions. Also two nuclei in protomerite. 42.5 by 11.25μ.

Fig. 9.—Free intestinal form showing no cuticle, 35.62 by 12.5μ.

Fig. 10.—Inside intestine lying close to epithelium. 30 by 5μ.

Fig. 11.—Conjugating pair attached to epithelial lining of intestine. Primite 56.25 by 10.6μ. Satellite 56.25 by 8.7μ.

Fig. 12.—Old pair of conjugants. Primite 62.5 by 15.62μ. Satellite 31.25 by 6.25μ.

Fig. 13.—Cyst found in intestine showing two sizes in the sporonts.

Fig. 14.—Sporozoites in intestinal lumen.

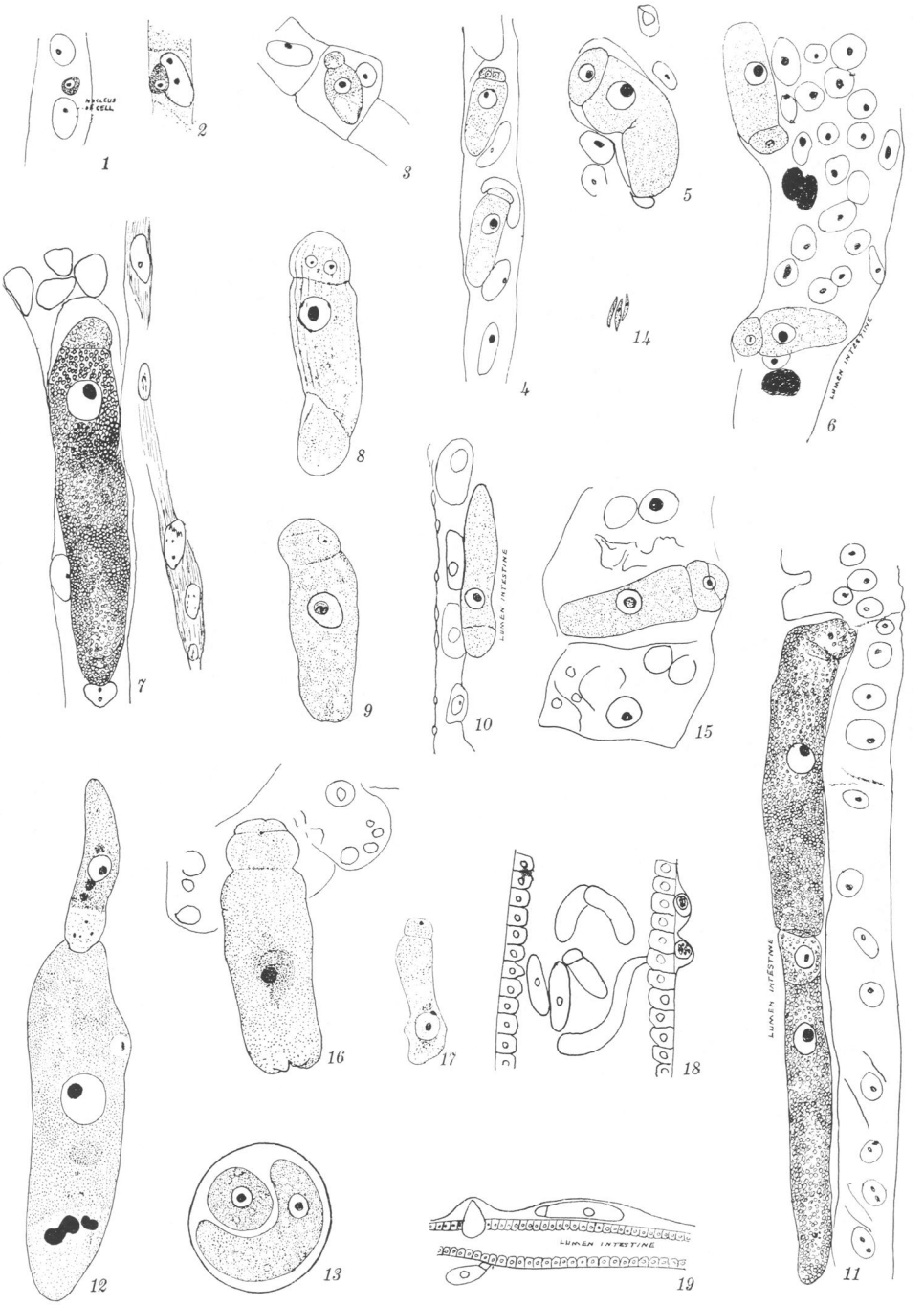
Fig. 15.—Young gregarine in liver tubule cell. 31.25 by 8.75μ.

Fig. 16.—Form protruding posterior end from a cell in hepatic cecum. Protomerite is constricted into a sort of epimerite. Nucleus is extruding chromatin. Posterior end of dentomerite invaginated. 43.75 by 18.75μ.

Fig. 17.—Shows chromatin extrusion. 25 by 6.25μ.

Fig. 18.—Diagrammatic sketch from living gregarines showing what is believed to be this form loose in the intestinal tract.

Fig. 19.—Diagrammatic sketch from living material showing the penetration of the intestinal epithelium by one gregarine, and the resting position of another just beneath the submucosa.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